Mouse Resources for conditional genetic manipulations

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Head of Experimental Animal Division
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The value of the mouse

- Both human and mouse are mammals
- Physiology, anatomy and metabolism parallel between human and mouse
- Whole-organ systems, physiological homeostasis, reproduction, behavior and disease
- Mouse genome encodes experimentally tractable organism
- Mice share 95% DNA coding sequence identity with humans
- We can determine the function of each gene by experimental manipulation and evaluation \textit{in vivo}
- Therefore, the mouse is an ideal tool for understanding human biology and disease processes
The mouse models human

Recent advancement of genetic manipulation in mice has enabled us to generate transgenic and knockout mice to understand human health and overcome diseases.

1. Studying the gene function *in vivo*
2. Human disease models
3. Discovering treatments and drugs

*Proc Natl Acad Sci U S A.* 1991 Dec 1;88(23):10885-10889. Dominant white spotting or piebald caused by a mutated KIT oncogene in both human and mouse. The mouse is a good animal model for human genetic diseases.
The 100 years’ history of the inbred mouse

Long history of laboratory mice

China
Japanese fancier

English fancier
Lathrop 1903-1915

Little 1909

Abbie Lathrop

Little Carnegie Institution

Lathrop 1903-1915

Little 1921

English fancier

Castle 1921

Tyzzer

Little 1929-30

Heston 1948

Russell 1945

Jax 1947

prior to 1937

Douglas 1928

Strong 1921

Strong 1927

Bittner 1928

Furth 1928-36

Rhoades 1936-40

Lynch 1940

Little 1921

MacDowell 1922

Snell 1935

Mishima 1979

Moriwaki

Dr. W.E. Castle
Dr. C.C. Little
Dr. L.C. Strong
Dr. Moriwaki

C57BL/6 Standard strain

129 ES cells

DBA/2 Acoustic seizure

C3H Mammary tumor

CBA Mammary tumor

A Lung tumor

AKR Leukemia

BALB/c Immunity

MSM Wild-derived
Why inbred?

Inbred mice are identical materials across time and space. The genetic uniformity ensures reproducible results like identical twins.

Inbreeding for more than 20 gen. results in uniform genome >99%.
Manipulating the mouse genome

The Nobel Prize in Physiology or Medicine 2007

http://www.nobelprice.org/

Gene targeting via ES cells

Mario R. Capecchi
Sir Martin J. Evans
Oliver Smithies

Nature 420, No.6915, 5 Dec 2002
We have generated various kinds of mice

- Inbred strain
- Spontaneous mutant
- Congenic strain
- Recombinant inbred and consomic strains
- ENU-induced mutant
- Transgenic mice
- Knockout mice
- Conditional knockout mice
- Cre- and Flp-mice
- Mice with an inducible switch for gene expression
- Reporters of gene expression/function
- Mutant mice by ZnF, Talen, CRISPR/Cas technologies
Cre-loxP site-specific recombination system

- A powerful tool for introducing engineered modifications into eukaryotic genomes.
- To study mammalian genome function
- To generate mouse models for human diseases.

Abbreviations

Cre: cyclization recombination
loxP: locus of X-over of P1
Cre-loxP: How it works?

Depends on the orientation and location of two loxP

A. Deletion

B. Inversion

C. Translocation
Improved properties of FLP recombinase evolved by cycling mutagenesis

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Received 17 December 1997; accepted 11 May 1998.

The site-specific recombinases FLP and Cre are useful for genetic engineering in many living systems. Manipulation of their enzymatic properties offers a means to improve their applicability to different host organisms. We chose to manipulate the thermostability of FLP recombinase. A lsoCB-based recombination assay in Escherichia coli was used for selection in a protein evolution strategy that relied on error-prone PCR and DNA shuffling. Improved FLP recombinases were identified that were capable of increasing the stability of FLP recombinase imposed by both raising temperature and reducing protein expression, combined with repetitive cycles of screening at the same stringency to enrich for clones with improved fitness. An eighth-generation clone (termed FLPe) showed improved properties in E. coli, in vitro, in human 293- and mouse ES-cells.

Keywords: molecular evolution; DNA shuffling; site-specific recombination

Applied site-specific recombination allows precise chromosome and transgene engineering in a broad range of living systems. FLP recombinase of the yeast Saccharomyces cerevisiae and Cre recombinase from the bacteriophage P1 are the two enzymes most often used in experiments to alter genetic material of an organism via site-specific recombination. These proteins have a range of applications, including chromosomal translocations and large deletions, tissue-specific and conditional knockout1, site-specific integration and inducible gene expression2, and precise removal of selectable markers. A number of applications based on the use of two highly active recombinases can be designed, for example, to manipulate double, homoplastic knockouts in vivo.

Our goal was to obtain an improved FLP recombinase that would allow inactivation by temperatures relevant to mammalian systems. Improvement of FLP by design is difficult because little information regarding structure of the protein is available. An effective strategy to alter protein properties involves creation of a selective environment in which the protein is successively optimized by a forced process of evolution, rather than by rational design. Evolutionary approaches do not carry any bias toward a specific preformed principle and can broadly and simultaneously improve many factors that contribute to the desired phenotype. Improvements that could lead to better recombination properties of FLP at elevated temperatures could include enhanced enzyme activity, increased protein stability, and protein mutants.

We randomly mutated the coding sequence of FLP and evaluated a screening protocol in E. coli that enriches for recombinases with improved fitness. Clones with higher fitness than wild-type FLP (FLPe) were collected in successive rounds of screening and recombination by DNA shuffling, which simultaneously combines positive mutations and removes negative mutations from the sequence pool. To address the possibility that the observed recombination improvements were dysgenic in E. coli, which grows at 30°C. Thus, FLP has no biological pressure to be active at higher temperatures. Indeed, FLP is active at 37°C, but its activity is not preserved by higher temperatures. However, we observed that inactivation of FLP recombinase was observed in E. coli, in vitro, in human 293- and mouse ES-cells.

Figure 4. Comparison of FLPe and FLPa recombination in mammalian cell culture. (A) The β-galactosidase FLP reporter cell line R10 transfected with either pOG-44wt or pOG-FLPe. Cells were incubated at indicated temperatures and stained with X-gal. (B) The β-galactosidase FLP reporter cell line a21 electrotransfected with either pOG-44wt or pOG-FLPe and stained with X-gal. (C) Cell extracts from R10 cells grown at 39°C with indicated plasmids assayed for β-galactosidase activity. For pflac-FLPe and pflac-FLPe, the measured values were multiplied by 10 for better presentation. Lane 1: pOG-44wt; lane 2: pOG-FLPe; lane 3: pOG-44wt; lane 4: pOG-FLPe; lane 5: pflac-FLPe; lane 6: pflac-FLPe.

FLPe: Thermostable FLP
Typical conditional knockout allele

- Delete neo and the 2\textsuperscript{nd} exon by crossing with Cre-deleter to generate tm1 null reporter allele
- Delete only marker genes and recover wild-type allele by crossing with Flp-deleter, and subsequently generate null allele without marker genes by crossing with Cre-deleter


**lacZ expression analysis and phenotyping**
Cre-loxP for reporter mice

A. Tg(CAG-CAT-Z) transgenesis

B. Knock-in lacZ reporter mouse
Need for spatiotemporal control of somatic mutagenesis in mice

- Early embryonic lethal analysis of gene functions in subsequent stages impossible
- Pleiotropy: many genes works in different cell types during embryonic and postnatal stages.
- Gene family members and their redundancy
- Impaired fertility and systemic diseases
- Human diseases such as cancer result from a combination of somatic mutations
International cooperation for Cre mice

The CREATE (Coordination of resources for conditional expression of mutated mouse alleles) project.

- The CREATE consortium represents a core of major European and international mouse database holders and research groups involved in conditional mutagenesis, primarily to develop a strategy for the integration and dissemination of Cre driver strains for modeling aspects of complex human diseases in the mouse.

- Collectively the participants have amassed a significant number of these strains in their respective databases as shown below. Therefore one of the goals of CREATE is to provide a unified portal for worldwide access to these critical resources.

http://www.creline.org/
Targeted conditional somatic mutagenesis defines an entirely new scientific field, driven by internationally coordinated initiatives (EUCOMM, KOMP, NorCOMM) established for the systematic generation of conditional mouse mutants on a large scale. The majority of these initiatives are committed to the production of mutant mouse ES cell lines, each of which carries an altered or "floxed" allele of a single gene. These mutant ES cell mutations can be readily transformed into mice using blastocyst injection, and the mutation activated by crossing the mouse bearing the floxed allele with a Cre recombinase driver strain to induce the mutation in spatially and temporally determined patterns.

Intricate conditional and inducible gene manipulation approaches have led to the generation of cell lineage- or developmental stage-specific alterations under temporal control. The vast number of Cre recombinase-expressing mouse lines - often referred to as "Cre-Zoo" animals - has greatly contributed to these accomplishments by allowing Cre recombinase to be expressed in specific cell types, in some cases in an inducible manner.

The full power of conditional mutant ES cell libraries and mice can therefore only be exploited with the availability of well characterized mouse lines expressing Cre-recombinase in tissue, organ and cell type-specific patterns, to allow the creation of somatic mutations in defined genes.
Methods for spatio-temporal control of recombination

- We need a tight external control of induction of recombination at a given time in a specific cell type.
- Use of ligand-dependent recombinases
- Engineering Cre + ligand binding domain (LBD) of steroid receptors (Cre + human estrogen receptor LBD)
- A problem in mice: Endogenous steroids
- Find mutants which is insensitive to endogenous estrogen and highly sensitive to synthetic estrogen antagonists
### Cre-ER variants and their activity

<table>
<thead>
<tr>
<th>Cre-ER</th>
<th>LBD-Type</th>
<th>No. of Amino acid in LBD.</th>
<th>beta-gal activity in F9 cells</th>
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<tr>
<td></td>
<td></td>
<td>400</td>
<td>521</td>
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<tr>
<td>Cre</td>
<td>none</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Cre-ER</td>
<td>GG (wild-type)</td>
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<td>G</td>
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<td>VG</td>
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<td>G</td>
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<tr>
<td>Cre-ER</td>
<td>VR</td>
<td>V</td>
<td>R</td>
</tr>
<tr>
<td>Cre-ER&lt;sup&gt;T&lt;/sup&gt;</td>
<td>GR</td>
<td>G</td>
<td>R</td>
</tr>
<tr>
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<td>AA1</td>
<td>V</td>
<td>G</td>
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<tr>
<td>Cre-ER&lt;sup&gt;T2&lt;/sup&gt;</td>
<td>AA2</td>
<td>V</td>
<td>G</td>
</tr>
</tbody>
</table>

ER: human estrogen receptor  
LBD: Ligand Binding Domain  
F9: The F9 mouse embryonal carcinoma cells  
E2: 17β-estradiol  
OHT: 4-hydroxytamoxifen  
ICI: ICI 182,780

Inducible reporter by CreER™

Tissue-Specific Promoter (TSP)  CreER™  pA

(+/-) Tamoxifen  activated

CreER™  (-) Tamoxifen

Not activated

TSP  STOP  Reporter
  loxp  loxp

TSP  STOP  Reporter
  loxp  loxp

TSP  Reporter

Reporter On

Reporter Off
**Cre-driver mice**

- Useful Cre mice for tissue-specific conditional experiments
- New Cre mice of C57BL/6N background generated by BRC program
- 128 Cre and 4 Flp mice available

**Search for Cre/Flp mice in BRC Web Catalog**

<table>
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<th>BRC No.</th>
<th>Status</th>
<th>Type</th>
<th>系統名</th>
<th>遺伝子</th>
<th>疾患モデル等</th>
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<td>Transgene</td>
<td>C57BL/6N-Tg(Ins1-cre)24Uttr/Rbrc</td>
<td>pre (Phage P1 Cre recombinase)</td>
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</table>

**Specificity:** Pancreas islet

**B6/N-Tg(Ins1-cre)**

*Genotype: Cre/+, lacZ/+*

**Specificity:** gut epithelium

**B6/N-Tg(Vil1-cre)**

*Small Intestine  Large intestine  Genotype: Cre/+, lacZ/+, inset: control*
Tissue-specific Cre mice
over 130 lines available for conditional experiments

**Ins1**
Pancreatic islets

**Ngn3**
spermatogonia
Pancreatic islets
Cerebral cortex
layer 4-6

**Emx1**
Cerebral cortex
Hippocampus

**Wap**
Mammary gland epithelial cells

**Tek/Tie2**
Blood vessels/Endothelial cells

**Vil1**
Gut epithelial cells
Cre expression analysis

A. FVB/N-Tg(P0-Cre)1Gth (RBRC01459) developed by Dr. Marco Giovannini.
B. B6.129P2-Emx1<sup>tm1(cre)lto</sup> (RBRC01345) developed by Dr. Shigeyoshi Itohara.
Both Cre mice were crossed with lacZ reporter mice, B6.129S4-Gt(ROSA)26Sor<sup>tm1Sor/J</sup> purchased from The Jackson Laboratory.
References for cre-loxP

TET system enables switch On/Off the gene expression

Temporal control of gene expression in transgenic mice by a tetracycline-responsive promoter

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Communicated by Philip Leder, May 5, 1994

ABSTRACT Promoters whose temporal activity can be directly manipulated in transgenic animals provide a tool for the study of gene functions in vivo. We have evaluated a tetracycline-responsive binary system for its ability to temporally control gene expression in transgenic mice. In this system, a tetracycline-controlled trans-activator protein (TAs), composed of the repressor of the tetracycline-resistance operon (tet from Escherichia coli transposon Tn10) and the activating domain of viral protein VP16 of herpes simplex virus, induces transcription from a minimal promoter (P<sub>CMMV</sub>-α) fused to seven tet operator sequences in the absence of tetracycline but not in its presence. Transgenic mice were generated that carried either a luciferase or a β-galactosidase reporter gene under the control of P<sub>CMMV</sub>-α or a transgene containing the TTA coding sequence under the control of the human cytomegalovirus immediate early gene 1 (hCMV IE1) promoter/enhancer. Whereas little luciferase or β-galactosidase activity was observed in tissues of mice carrying only the reporter genes, the presence of TTA in double-transgenic mice induced expression of the reporter genes up to several thousand-fold. This induction was abrogated to basal levels upon administration of tetracycline. These findings can be used, for example, to design dominant gain-of-function experiments in which temporal control of transgene expression is required.

85% of the world leading pharmaceutical companies use the Tet Technology
Components of TET system

A: tTA

B: rtTA

C: P_{tet} -1

D: Doxycycline (Dox)
tTA: tetracycline-controlled transactivator

- **tTA** is a hybrid transcription factor
  - The prokaryotic Tet repressor, TetR + an eukaryotic transcriptional transactivation domain (the acidic domain of HSV VP16).
  - The TetR moiety confers sequence specific DNA binding, sensitivity to tetracyclines and dimerization to the tTA fusion protein.
  - Accordingly, the response of both TetR and tTA to tetracyclines is similar: binding of the antibiotic dramatically lowers their affinity to their common cognate binding sites, the tet operators.
rtTA differs from tTA by a few point mutations (\*) within TetR.  
- The mutations result in a complete reversal of tetracycline responsiveness of this transcription factor.  
- rtTA requires tetracyclines for binding to tetO.  
- Specific tetracycline derivatives like doxycycline (Dox) or anhydrotetracycline (ATc) must be used to optimally exploit the rtTA phenotype.
\( P_{\text{tet}} \): a synthetic TET promoter

\( P_{\text{tet}} \) is a synthetic promoter responsive to both tTA and rtTA.

- It is comprised of a minimal RNA polymerase II promoter fused to multimerized \( tetO \) sequences. This arrangement makes the activity of \( P_{\text{tet}} \) dependent on the binding of tTA or rtTA.
- The original version which consists of a CMV minimal promoter fused to an array of seven \( tetO \) sequences is designated \( P_{\text{tet}}-1 \).
Doxycycline, a tetracycline derivative, is currently the most preferable effector substance for both the Tet-On and the Tet-Off System.

- It binds with high affinity to tTA as well as to rtTA and, thus, is fully effective in the Tet-Off system at concentrations as low as 1-2 ng/ml in the case of tTA.
- An excellent medical safety record and well characterized pharmacological properties like excellent tissue penetration and low toxicity in eukaryotes.
- Doxycycline hydrochloride (Dox-HCl), which, like tetracycline hydrochloride, is water soluble.
Tet-Off system: How does it work?

In the presence of Dox (+), the gene X is inactive (Off).
In the absence of Dox (-), the gene X is activated (On).
Tet-On system: How does it work?

In the presence of Dox (+), the gene X is activated (Off).
In the absence of Dox (-), the gene X is inactive (On).
How to increase the site-specificity?

You can control gene expression in the restricted area in Yellow.

Temporal control by TET-Off system

\[ t_0 \text{ Dox}(-) \quad \text{X-On} \]
\[ t_1 \text{ Dox}(+) \quad \text{X-Off} \]
\[ t_2 \text{ Dox}(-) \quad \text{X-On} \]
Models for the renal function and disease

1) Polycystic kidney disease and renal cancer: 
Pax8-rtTA X P_{tet}-MYC (a, b, c, d)

2) Renal fibrosis by overexpression of Tgf-β1: 
Pax8-rtTA X P_{tet}-Tgf-b1 (e, f)

3) Severe polycystic kidney disease: 
Pax8-rtTA X P_{tet}-cre X Tsc1^{flox/lox} (g, h)
To study brain functions


Test the mice in maze for learning and memory
References for TET


Invitation to

Mouse of the Month


for conditional tools
May 2013
Mouse of the Month
Regulating gene expression in photoreceptor precursor cells

B6.Cg-Tg(Cox-cre/ERT2)1Tfut/TfutRbrc; RBRC05669

X-gal staining of sections isolated from a Cox-cre/ERT2, CAG-GAT-Z reporter with tamoxifen.
Application of tamoxifen induced Cre-mediated recombination of the LacZ reporter transgene in the developing photoreceptor layer of the CAG-GAT-Z reporter mouse (provided by Dr. J. Myazaki, Osaka University). ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.

Cone-rod homeobox containing gene (CRX) is a transcription factor that is predominantly expressed in developing and mature photoreceptor cells in the retina, and plays a crucial role in the terminal differentiation of rods and cones. CRX is one of the earliest expressed genes in photoreceptor precursor cells [1-4]. To obtain the regulation of Cre expression in photoreceptor precursor cells, Furukawa and colleagues established a transgenic mouse line (Cox-CreERT2) that expresses CreERT2 under the control of a 2-kb promoter fragment from the Cox gene [5, 6]. Application of tamoxifen to Cox-CreERT2; CAG-GAT-Z indicator mice induced Cre-mediated recombination of a LacZ reporter transgene in the developing photoreceptor layer. They also inactivated the retina and anterior neural fold homeobox (Rax) gene in early postmitotic photoreceptor precursor cells using the CreERT2 system and revealed that the expression of the transcription factor orthodenticle homolog 2 (Otx2) was severely attenuated in the Rax-induced conditional knockout retina [6]. Cox-CreERT2 transgenic mice allow us to regulate gene expression in photoreceptor precursor cells from the embryonic to the adult stage.

Jun 2013
Mouse of the Month
A tool for the conditional deletion of the transcriptional repressor Bcl6

B6.Cg-Blc6floxed1/?Rbrc; RBRC05663

Histological distribution of IgG1+ memory B cells in the spleens of conditional Bcl6-deficient (Bcl6?/?) mice (mbrcre+/+), control (Bcl6?) and (mbrcre+/+) mice. Memory B cells were identified by their expression of peanut agglutinin (PNA) (yellow) and IgG1 (green) on their cell surface. The Bcl6 knockout mice were 2 weeks old, and the control mice were 20 weeks old. Memory B cells were detected in the peritoneal cavity (data not shown).

Memory B cells are long-lived quiescent B cells selected for their expression of somatically mutated, high-affinity antibodies in the B cell-dependent germinal center (GC) reaction. This reaction is accompanied by the up-regulation of the transcriptional repressor B cell lymphoma/involution (Bcl6), on which GC B cell differentiation depends. A puzzling observation has been that the memory B cell compartment also contains cells expressing unmutated, low-affinity antibodies. To obtain a comprehensive understanding of the population dynamics underlying GC-dependent and -independent memory B cell development, Takemori and colleagues established a mutant mouse strain carrying a loxP-flanked Bcl6 exon 7-8 allele, with these exons encoding the Bcl6 zinc finger domains ZF1 to ZF5. Using conditional Bcl6 ablation, they demonstrated that the unmutated memory cells are generated through proliferative expansion early after immunization in a B cell-dependent manner. GC-independent memory B cells are otherwise induced, and both types of memory cells efficiently generate an adoptive secondary antibody response [1].

Related strains: 129.B6G3-Tg(Cox-cre)1Tfut/TfutRbrc; RBRC05428

Depositor: Toshitada Takemori, M.D., Ph.D., RIKEN Center for Integrative Medical Sciences
Macrophages and alveolar epithelial type II (AE2) cells express the lysozyme M (LysM) gene. B6.129-Lyz2tm1(DTR).MtkBam (LysM-DTR) mice were generated by targeting the LysM gene with a construct containing the human diptheria toxin (DT) receptor (DTR) and heparin-binding epidermal growth factor-like growth factor in exon 1 of LysM. This transgenic mouse can serve as a toxin receptor-mediated conditional cell knockout model of acute respiratory distress syndrome by specifically deleting AE2 cells and alveolar macrophages after DT treatment. When DT is administered to these mice, they suffer from acute lung injury and die within 4 days. The amount of surfactant proteins in bronchoalveolar lavage fluid is significantly decreased in these DT-treated LysM-DTR mice. A transplantation of wild-type bone marrow cells to irradiated LysM-DTR mice can restore macrophages resistant to DT, thereby selectively deleting AE2 cells by DT administration. These conditional models are useful to clarify the roles of macrophages and AE2 cells in non-inflammatory lung injury.

Depositor: Dr. Masato Tanaka, RIKEN Research Center for Allergy and Immunology

Cre reporter mouse expressing nuclear-localized beta-galactosidase

E6.129P2-Crt(Rosa26Sor<tm1(NLS-lacZ)1to>/IoRbrc RBRC02657

R26 reporter

NLS-lacZ reporter

Courtesy of Dr. Shintaro Ishihara, RIKEN BSI

Cre recombinase is a 38-kDa enzyme derived from bacteriophage P1 that specifically recognizes 34-bp loxP sites. Cre is among the most widely used site-specific recombinases in genome engineering. For identifying the derivatives of Cre-reporting cells, a number of reporter mouse strains have been reported, such as ROSA26 reporter mice (R26R mice) [1]. One potential disadvantage of these Cre-reporter mice is that analysis at the level of single cells can be difficult or impossible in neuronal tissue (e.g., cerebral cortex and thalamus), due to diffuse signals in the cell cytoplasm after staining.

Dr. Ishihara and colleagues targeted the ROSA26 locus to generate new reporter mice (R26-NLS-lacZ or RNZ mice) in which lacZ, including the SV40 nuclear localization signal (NLS) was inserted downstream of the loxP-flanked PGK-neo cassette [2]. The left two panels show X-gal staining of coronal brain sections from Emx1-Cre/R26R mice. Cre-mediated recombination in Emx1-Cre mice (RBRC01945) is reportedly specific to excitatory cortical neurons (3, 4). The two right panels show sections from Emx1-Cre/RNZ mice indicating that RNZ reporter mice expressing nuclear-localized beta-galactosidase allow for single-cell resolution. The new RNZ mice are useful as a Cre reporter strain following Cre-mediated recombination.

Related strains: Emx1-Cre Kl mice

E6.129P2-Emx1<tm1.1(Cre)Jo>/IoRbrc RBRC01345

A standard Flip dye marker

CS7EL/6-Tg(CAG-FLPeRbrc RBRC01834

April 2013

Mouse of the Month

Expanding the repertoire of optogenetic control

E6.129B6(Cg)-Actb<tm1(tetO-ChR2-C128S/EYFP)Kftnko/KftnRbrc RBRC004154

nCaMKII-tTA (Mayford, 1999)

KENG-tet

tetO cassette insertion into p-acin locus

knockin (RB05454)

BAC Tg (RB05453)

Iba1-tTA (RB05676)

nCaMKII-tTA (Mayford, 1999)

KENG-tet

tetO cassette insertion into p-acin locus

knockin (RB05454)

BAC Tg (RB05453)

Iba1-tTA (RB05676)

nCaMKII-tTA (Mayford, 1999)

KENG-tet

tetO cassette insertion into p-acin locus

knockin (RB05454)

BAC Tg (RB05453)

Iba1-tTA (RB05676)

KENG-tet enables us to achieve sufficient opsin expression to trigger photocurrents in combination with existing cell-type-specific tTA lines. All pictures show tTA-mediated ChR2-EYFP induction by KENG-tet.

Optogenetic strategies for controlling neuronal activity have been employed widely for mapping functional circuits within the nervous system. Channelrhodopsin-1 (ChR1) and ChR2, related chlorophyll-type rhodopsins from Chromatium vinosum and Chlamydomonas reinhardtii, are invoked in the generation of photocurrents. ChR2 is a light-gated cation channel, whereas ChR1 is a light-gated proton channel. The K-terminal 315 amino acids of ChR2 are homologous to the seven trans-membrane structure of numerous microbial-type rhodopsins. Inward currents in ChR2-expressing cells can be evoked after a flash of blue light in the presence of all-trans retinal [1, 2].

Dioscori: Kenji Tanaka, M.D., Ph.D.
Department of Neurosurgery, Keio University School of Medicine
Akihiko Yamashita, Ph.D.
Research Institute of Environmental Medicine, Nagoya University

References:
February & March 2013
Mouse of the Month
Multifunctional Rosa26 reporter mouse strain
E6.129S6-Gt(ROSA)26Sor-tm1(CAG-mTFP)Jmav0/JmavObrc ERBC05146

Schematic illustration of the Cre- and/or Flp-dual recombinase responsive alleles. A transgenic cassette composed of an SA (spliced acceptor sequence)–promoter–huromycin resistance gene, the CAG promoter, a floxed 3xSV40SA stop cassette (orange triangle), a 3x PA (SV40poly-TAGA-SV40polyPA) stop cassette flanked by FRT sites (blue triangles), effector gene (green box), and Flpe–SV40PA was inserted in the ROSA26 locus. R26-CAG-LF-Effectort was modified by the germline excision of the FRT-flanked stop cassette using the pCAG-FLP mouse to produce a Cre-dependent effector line (R26-CAG-LoxP-Effectort). The germline excision of the floxed stop cassette using the TNAP-Cre mouse converts the R26-CAG-LF-Effectort line to a Flp-dependent effector line (R26-CAG-FRT-Effectort).

June 2012
Mouse of the Month
Atg7 deletion in various tissues
E6.129C6-Bfl(1)Tc[r]Tcbrc ERBC02759
E6.129C6-Bfl(1)Tc[r]Tcbrc ERBC02760

Generation of tissue-specific Atg7-deficient mice

Autophagy fights various diseases

To investigate the physiological roles of autophagy in mice, conditional knockout mice for an autophagy-essential gene, Atg7, were generated. A number of groups have generated various tissue-specific autophagy-deficient mice. Many of the molecular mechanisms underlying autophagy and the physiological roles of these processes have since been elucidated.

Autophagy is a pathway for bulk protein degradation that has been conserved in most eukaryotic cells. The initial steps of autophagy include the formation and elongation of the isolation membrane. This membrane encloses organelles and other cytoplasmic components until forming a double-membrane vesicle called an autophagosome. The biogenesis of autophagosomes requires proteins encoded by autophagy-related (Atg) genes, most of which were initially identified in yeast. To date, Atg8 conditional knockout mice, GFP-tagged Man6p tags Tip mice (man66 homologues of Atg8), and other various Atg-deficient mice have been established [1].

While autophagy has been considered a non-selective process, growing evidence indicates that selectivity of autophagy plays an important role in the turnover of aberrant proteins, disposal of damaged organelles, and elimination of protein aggregates and invading pathogens. Tissue-specific Atg7-deficient mice exhibit liver injury, diabetes, muscle atrophy, and neurodegeneration [2–4]. Conventional and conditional Atg7 knockout mice provide the opportunity to understand the role of autophagy in homeostasis and various common diseases.

Related strain: E6.129C6-Bfl(1)Tc[r]Tcbrc ERBC02759

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Thank you for your attention

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